PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

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PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	22 JUL 2008
Applicant's or agent's file reference DMB-4112-72	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 08/04708	11 April 2008 (11.04.2008)	13 April 2007 (13.04.2007)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C07C 57/00; C11B 3/00; C07C 51/00 (2008.04)
USPC - 554/224; 554/124; 554/156

Applicant DIFFUSION PHARMACEUTICALS LLC

1. This opinion contains indications relating to the following items:

- [x] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [x] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	10 July 2008 (10.07.2008)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (April 2007)

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Box No. 1 Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this opinion has been established on the basis of:

   a. type of material

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material

      ☐ on paper

      ☐ in electronic form

   c. time of filing/furnishing

      ☐ contained in the international application as filed

      ☐ filed together with the international application in electronic form

      ☐ furnished subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

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Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	2-4, 7-8, 14, 18, 20, 22-25, 27, 29, 31-33, 36	YES
	Claims	1, 5-6, 9-13, 15-17, 19, 21, 26, 28, 30, 34-35	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-36	NO
Industrial applicability (IA)	Claims	1-36	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1, 5-6, 9-13, 15-17, 19, 21, 26, 28, 30, 34 and 35 lack novelty under PCT Article 33(2) as being anticipated by US 7,145,025 B2 to Lockwood et al. (hereinafter 'Lockwood').

Regarding claim 1, Lockwood teaches the method of treating a mammal ("a method for inhibiting and/or ameliorating the occurence of diseases associated with reactive oxygen species" - Abstract) having peripheral vascular disease ("peripheral vascular disease" - col 4, ln 58-59) comprising:
-- administering a therapeutically effective amount of a trans carotenoid ("water soluble analogs of carotenoids may be administered to a subject" - col 6, ln 43-45; "disodium salt disuccinate astaxanthin derivative" - Fig. 17).

Regarding claim 5, Lockwood teaches the method of reducing the formation of atherosclerotic plaque in a mammal having atherosclerotic plaque ("a method for inhibiting and/or ameliorating the occurence of diseases associated with reactive oxygen species" - Abstract; "artherosclerosis" - col 4, ln 58) comprising:
-- administering to said mammal a therapeutically effective amount of a bipolar trans carotenoid ("water soluble analogs of carotenoids may be administered to a subject" - col 6, ln 43-45; "disodium salt disuccinate astaxanthin derivative" - Fig. 17).

Regarding claim 6, Lockwood teaches the method of reducing damage due to an ischemic event in a mammal at risk of an ischemic event ("a method for inhibiting and/or ameliorating the occurence of diseases associated with reactive oxygen species" - Abstract; "ischemia-reperfusion damage" - col 6, ln 7-8) comprising
-- administering to said mammal a therapeutically effective amount of a trans carotenoid ("water soluble analogs of carotenoids may be administered to a subject" - col 6, ln 43-45; "disodium salt disuccinate astaxanthin derivative" - Fig. 17).

Regarding claim 9, Lockwood further teaches the method of claim 6, in which said mammal at risk of an ischemic event is a mammal at risk of stroke ("stroke" - col 4, ln 50).

Regarding claim 10, Lockwood further teaches the method of claim 6, in which said mammal at risk of an ischemic event is a mammal at risk of forming an embolism or blood clot ("thromboembolic" - col 4, ln 50).

Regarding claim 11, Lockwood further teaches the method of claim 6, in which said mammal at risk of an ischemic event is a mammal at risk of significant blood loss ("hemorrhagic" - col 4, ln 50-51).

Regarding claim 12, Lockwood further teaches the method of claim 6, in which said mammal at risk of an ischemic event is a mammal with constricted arteries ("arterial occlusion" - col 6, ln 25).

Regarding claim 13, Lockwood teaches a method of treating a mammal having angina ("a method for inhibiting and/or ameliorating the occurence of diseases associated with reactive oxygen species" - Abstract; "chronic stable angina, unstable angina" - col 6, ln 7-8) comprising
-- administering a therapeutically effective amount of a trans carotenoid ("water soluble analogs of carotenoids may be administered to a subject" - col 6, ln 43-45; "disodium salt disuccinate astaxanthin derivative" - Fig. 17).

Regarding claim 15, Lockwood teaches a method of treating macular degeneration ("a method for inhibiting and/or ameliorating the occurence of diseases associated with reactive oxygen species" - Abstract; "aged-related macular degeneration" - col 4, ln 36-37) comprising
-- administering a therapeutically effective amount of a trans carotenoid ("water soluble analogs of carotenoids may be administered to a subject" - col 6, ln 43-45; "disodium salt disuccinate astaxanthin derivative" - Fig. 17).

Regarding claim 16, Lockwood further teaches the method of claim 1, in which the bipolar trans carotenoid is administered by intravenous administration ("water soluble analogs of carotenoids may be administered to a subject" - col 6, ln 43-45; "disodium salt disuccinate astaxanthin derivative" - Fig. 17; "suitable solutions for administration by injection" - col 57, ln 59-60).

Regarding claim 17, Lockwood further teaches the method of claim 5, in which the bipolar trans carotenoid is administered by oral administration ("preparations which may be administered orally" - col 57, ln 54-55).

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box No. V (2) Citations and Explanations:

Regarding claim 19, Lockwood further teaches the method of claim 5, in which said bipolar trans carotenoid is in the form of a composition comprising a trans carotenoid and a cyclodextrin ("cyclodextrins" - col 59, ln 25-26).

Regarding claim 21, Lockwood further teaches the method of claim 19, in which the cyclodextrin is beta cyclodextrin ("beta cyclodextrin" - col 59, ln 26).

Regarding claim 26, Lockwood further teaches the method of claim 5, in which the trans carotenoid is a salt ("disodium salt disuccinate astaxanthin derivative" - Fig. 17).

Regarding claim 28, Lockwood further teaches the method of claim 5, in which the trans carotenoid is a acid ("disodium salt disuccinate astaxanthin derivative" - Fig. 17).

Regarding claim 30, Lockwood further teaches the method of claim 19, further comprising mannitol ("mannitol" - col 58, ln 12).

Regarding claims 34-35, Lockwood further teaches the method of claim 19, in which said composition is lyophilized ("lyophilizing processes" - col 58, ln 1).

Claims 2-4 and 7-8 lack an inventive step under PCT Article 33(3) as being obvious over Lockwood, as above.

Regarding claim 2, Lockwood teaches the method of claim 1, in which the disease is peripheral vascular disease. Lockwood does not disclose the peripheral vascular disease is specifically peripheral arterial disease. However, Lockwood discloses the use of carotenoids for the further treatment of venous or arterial occlusion (col 6, ln 25) and ischemia-reperfusion damage (col 6, ln 7-8). Since arterial occlusion can lead to ischemia-reperfusion damage, it would have been obvious to one skilled in the art to apply Lockwood's method of claim 1 specifically on peripheral arterial disease.

Regarding claims 3 and 4, Lockwood teaches the method of claim 1, in which the disease is peripheral vascular disease. Lockwood does not specifically disclose the ABI of the human subject is less than 1 such as 0.6. However, since ABI is a measure of the fall in blood pressure in the arteries supplying the legs, it would have been obvious to one skilled in the art to apply Lockwood's method of claim 1 in order to raise the ABI above 1, thereby treating the subject's peripheral vascular disease.

Regarding claim 7, Lockwood teaches the method of claim 6, in which said mammal is at risk of an ischemic event such as stroke ("a method for inhibiting and/or ameliorating the occurence of diseases associated with reactive oxygen species" - Abstract; "ischemia-reperfusion damage" - col 6, ln 7-8; "stroke" - col 4, ln 50). Lockwood does not specifically disclose the subject has no symptoms relating to ischemia. However, since Lockwood discloses inhibiting the occurrence of a disease such as stroke, it would have been obvious to one skilled in the art to treat a mammal at risk of an ischemic event even though no ischemia-related symptoms are observed in order to mitigate the risk by prophylactic treatment before permanent damage occurred.

Regarding claim 8, Lockwood teaches the method of claim 6, in which said mammal is at risk of an ischemic event such as stroke ("a method for inhibiting and/or ameliorating the occurence of diseases associated with reactive oxygen species" - Abstract; "ischemia-reperfusion damage" - col 6, ln 7-8; "stroke" - col 4, ln 50). Lockwood does not specifically disclose the subject has a blockage in a carotid artery. However, Lockwood discloses the treating arterial occlusion (col 6, ln 25). Since an ischemic event such as stroke can result from arterial occlusion in the carotid artery, it would have been obvious to one skilled in the art to apply Lockwood's method of claim 6 on a subject with a blockage in a carotid artery in order to treat the arterial occlusion before an ischemic event such as stroke occurs.

Claims 14, 18, 20, 22-25, 27, 29, 31-33, and 36 lack an inventive step under PCT Article 33(3) as being obvious over Lockwood, as above, in view of WO 2006/104610 A2 to Gainer et al. (hereinafter 'Gainer').

Regarding claim 14, Lockwood discloses the use of trans carotenoids to treat ischemia-reperfusion damage (col 6, ln 7-8); and Gainer discloses the use of trans carotenoids to increase cellular oxygen levels (pg 27, Fibromyalgia). Lockwood and Gainer do not specifically diclose the use of trans carotenoids to treat ischemic osteonecrosis. However, since osteonecrosis is caused by cutting off blood flow and oxygen to bone cells, it would have been obvious to one skilled in the art to combine Lockwood's treatment of ischemia and Gainer's increasing cellular oxygen in order to replenish oxygen levels in bone tissue that has experienced ischemia.

Regarding claim 18, Lockwood teaches the method of claim 5. Lockwood does not specifically disclose further administering oxygen to said mammal. However, Gainer teaches the administration of trans carotenoids to avoid oxygen deprivation during labor ("during labor to avoid oxygen deprivation" - pg 27). Since atherosclerosis taxes the heart which in turn taxes the lungs to supply more oxygen, it would have been obvious to one skilled in the art to administer oxygen and the trans carotenoid in order to achieve a greater effect in oxygenating the patient.

Regarding claim 20, Lockwood teaches the method of claim 19. Lockwood does not specifically disclose the cyclodextrin is alpha cyclodextrin. However, Gainer discloses cyclodextrins such as alpha cyclodextrin for solubilizing trans carotenoids ("alpha, beta, and gamma cyclodextrins" - pg 20). Since cyclodextrins are well-known in the art to solubilize active pharmaceutical ingredients, it would have been obvious to one skilled in the art to experiment with alpha cyclodextrin as an excipient in order to find a suitable excipient that would increase the bioavailability and pharmacokinetic properties of the trans carotenoid composition.

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box V(2) and the preceding Supplemental Box:

Regarding claim 22, Lockwood teaches the method of claim 19. Lockwood does not specifically disclose the cyclodextrin is 2-hydroxylpropyl-beta-cyclodextrin. However, Gainer discloses 2-hydroxylpropyl-beta-cyclodextrin for solubilizing trans carotenoids (pg 78, Claim 16). Since cyclodextrins are well-known in the art to solubilize active pharmaceutical ingredients, it would have been obvious to one skilled in the art to experiment with 2-hydroxylpropyl-beta-cyclodextrin as an excipient in order to find a suitable excipient that would increase the bioavailablity and pharmacokinetic properties of the trans carotenoid composition.

Regarding claim 23, Lockwood teaches the method of claim 19. Lockwood does not specifically disclose the cyclodextrin is gamma cyclodextrin. However, Gainer discloses gamma cyclodextrin for solubilizing trans carotenoids (pg 78, Claim 17). Since cyclodextrins are well-known in the art to solubilize active pharmaceutical ingredients, it would have been obvious to one skilled in the art to experiment with gamma cyclodextrin as an excipient in order to find a suitable excipient that would increase the bioavailablity and pharmacokinetic properties of the trans carotenoid composition.

Regarding claim 24, Lockwood teaches the method of claim 19. Lockwood does not specifically disclose the cyclodextrin is 2-hydroxylpropyl-gamma-cyclodextrin. However, Gainer discloses 2-hydroxylpropyl-gamma-cyclodextrin for solubilizing trans carotenoids (pg 78, Claim 18). Since cyclodextrins are well-known in the art to solubilize active pharmaceutical ingredients, it would have been obvious to one skilled in the art to experiment with 2-hydroxylpropyl-gamma-cyclodextrin as an excipient in order to find a suitable excipient that would increase the bioavailablity and pharmacokinetic properties of the trans carotenoid composition.

Regarding claim 25, Lockwood teaches the method of claim 5. Lockwood does not specifically disclose the trans carotenoid is crocetin. However, Gainer teaches the trans carotenoid, crocetin (pg 77, Claim 9). Since carotenoids are anti-oxidants, it would have been obvious to one skilled in the art to use a trans carotenoid such as crocetin in order to trap reactive oxygen species which are involved in the pathology of atherosclerosis.

Regarding claim 27, Lockwood teaches the method of claim 5. Lockwood does not specifically disclose the trans carotenoid salt is TSC. However, Gainer teaches the trans carotenoid salt, TSC (pg 7). Since carotenoids are anti-oxidants, it would have been obvious to one skilled in the art to use a trans carotenoid salt such as TSC in order to trap reactive oxygen species which are involved in the pathology of atherosclerosis.

Regarding claim 29, Lockwood teaches the method of claim 5. Lockwood does not specifically disclose the trans carotenoid is crocin. However, Gainer teaches the trans carotenoid, crocin (pg 77, Claim 13). Since carotenoids are anti-oxidants, it would have been obvious to one skilled in the art to use a trans carotenoid such as crocin in order to trap reactive oxygen species which are involved in the pathology of atherosclerosis.

Regarding claim 31, Lockwood teaches the method of claim 19. Lockwood does not specifically disclose further comprising saline. However, Gainer discloses administration of TSC in saline (pg 22, para 1). Since saline is a safe solution for intravenous administration, it would have been obvious to one skilled in the art to administer the trans carotenoid composition of claim 19 in a saline solution in order to safely dilute the composition and prepare an isotonic solution for intravenous administration.

Regarding claims 32-33, Lockwood teaches the method of claim 19. Lockwood does not specifically disclose further comprising a compound to regulate the pH selected from glycine. However, Gainer teaches adding a buffer such as glycine to trans carotenoid (pg 22, Buffers). Since different protonated forms of compounds have different stabilities, it would have been obvious to one skilled in the art to buffer Lockwood's trans carotenoid composition with glycine in order to form a stable composition in which the phamacokinetic properties are constant.

Regarding claim 36, Lockwood teaches the method of claim 5. Lockwood does not specifically disclose the bipolar trans carotenoid is TSC. However, Gainer teaches the bipolar trans carotenoid, TSC (pg 7). Since carotenoids are anti-oxidants, it would have been obvious to one skilled in the art to use a trans carotenoid such as TSC in order to trap reactive oxygen species which are involved in the pathology of atherosclerosis.

Claims 1-36 have industrial applicability as defined in PCT Article 33(4) because the subject matter can be made or used in industry.